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Molecules in focus

EGF receptor

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Abstract

The receptor for the epidermal growth factor (EGF) and related ligands (EGFR), the prototypal member of the superfamily of receptors with intrinsic tyrosine kinase activity, is widely expressed on many cell types, including epithelial and mesenchymal lineages. Upon activation by at least five genetically distinct ligands (including EGF, transforming growth factor-α (TGFα) and heparin-binding EGF (HB-EGF)), the intrinsic kinase is activated and EGFR tyrosyl-phosphorylates itself and numerous intermediary effector molecules, including closely-related c-erbB receptor family members. This initiates myriad signaling pathways, some of which attenuate receptor signaling. The integrated biological responses to EGFR signaling are pleiotropic including mitogenesis or apoptosis, enhanced cell motility, protein secretion, and differentiation or dedifferentiation. In addition to being implicated in organ morphogenesis, maintenance and repair, upregulated EGFR signaling has been correlated in a wide variety of tumors with progression to invasion and metastasis. Thus, EGFR and its downstream signaling molecules are targets for therapeutic interventions in wound repair and cancer. Published by Elsevier Science Ltd.

Keywords: Receptor protein tyrosine kinase (RPTK); Tumor invasion; Wound healing; Organogenesis; Signaling pathways

1. Introduction

Cell surface molecules communicate information from the external milieu to the cell. This sensing is critical in multicellular organisms as the cells must function appropriately to their localization and respond in concert to the needs of the organism. One major family of sensors is comprised of transmembrane receptors with intrinsic protein tyrosine kinase activity (RPTK), the prototypal member of which is the EGF receptor (EGFR; also referred to as HER (human EGF receptor) and c-erbB1) as it was the first receptor described to possess tyrosine kinase activity and the first member of this superfamily to be sequenced. Co-incidentally, the structure of EGFR appears to represent an archetypal pattern for this superfamily of extracellular sensors that control basic cell functions.

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[1] I apologize to all authors whose important works contributed to our understanding of the EGF receptor but could not be directly cited in this review due to the limitations in references.

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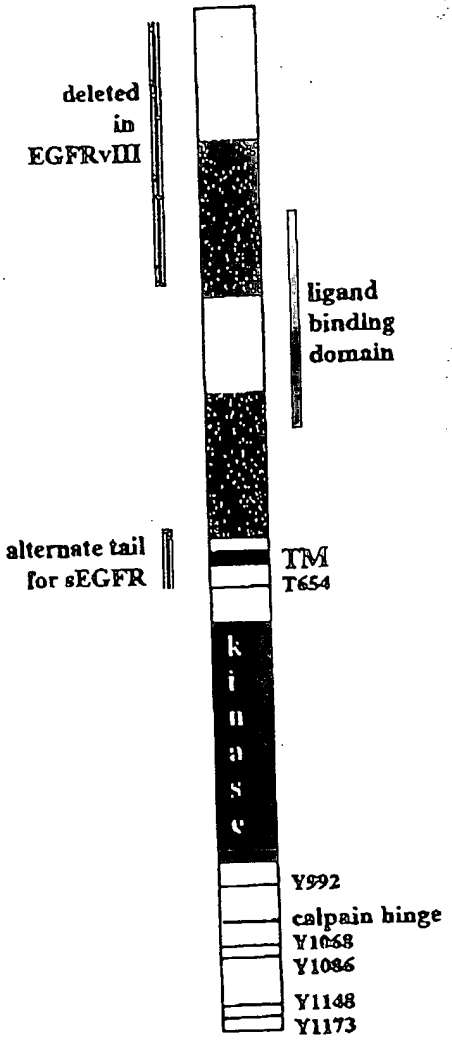

Fig. 1. Structural motifs and regulatory elements in the EGF receptor. The mature EGFR polypeptide is shown. On the left changes which characterize the two variants are shown: the deletion of exons 2–7 in EGFRvIII and the alternatively spliced tail of the secreted EGFR (sEGFR). Other highlighted structures include the two cysteine-rich (CR) domains, the discontinuous ligand-binding domains, which are different but overlapping for the various ligands. The transmembrane (TM) stretch separates the glycosylated extracellular domain from the intracellular regions. This latter includes the tyrosine kinase domain as well as the autophosphorylated tyrosines (Y), the site of PKC transmodulation on threonine at amino acid 654 (T654), and the calpain cleavage site. Not shown are the three internalization domains (at 973, 996, and 1149).

These receptors all present kinase activity directed against tyrosine residues located both within the receptor itself (autophosphorylation) and on target downstream molecules. Ligand binding activates the kinase which, with a possible few minor exceptions, is required for all cellular responses. The pleiotropic cell responses, actuated via still ill-defined pathways, include cell proliferation, migration, and differentiation as well as homeostatic functioning.

2. Structure

EGFR is somewhat unusual among RPTK in that there is a single isoform, from a single 26 exon gene located across 110kb on chromosome 7p11–13, which serves as the sole or overwhelmingly predominant receptor for multiple distinct ligands including EGF, TGFα, amphiregulin, HB-EGF and a number of virally-encoded factors. The protein product of this gene is most often an 1186 amino acid mature transmembrane glycoprotein (Fig. 1). An amino-terminal 622 amino acid extracellular domain containing two cysteine-rich domains comprises the ligand binding domain. There is a single alpha-helical transmembrane pass. The intracellular 542 amino acids can be grouped into three domains. The juxtamembrane domain (~50 amino acids) serves primarily as a site for feedback attenuation by PKC (protein kinase C) and erk MAP kinases (extracellular signal-regulated kinase, mitogen-activated protein kinase), though there is evidence that a motif within this region may link to heterotrimeric G proteins [1]. Next comes a contiguous ~250 amino acid tyrosine kinase (SH1, src homology 1) domain. A unique 229 amino acid long carboxy-terminal tail contains five autophosphorylation motifs which link to proteins containing SH2 or PTB (phospho-tyrosine binding) domains, at least three internalization motifs comprised of a tight turn, and sites for transphosphorylation and proteolytic activation and degradation. This tail also functions as an autoinhibitory substrate; in the absence of either autophosphorylation or removal, ligand-activated EGFR is unable to phosphorylate substrates.

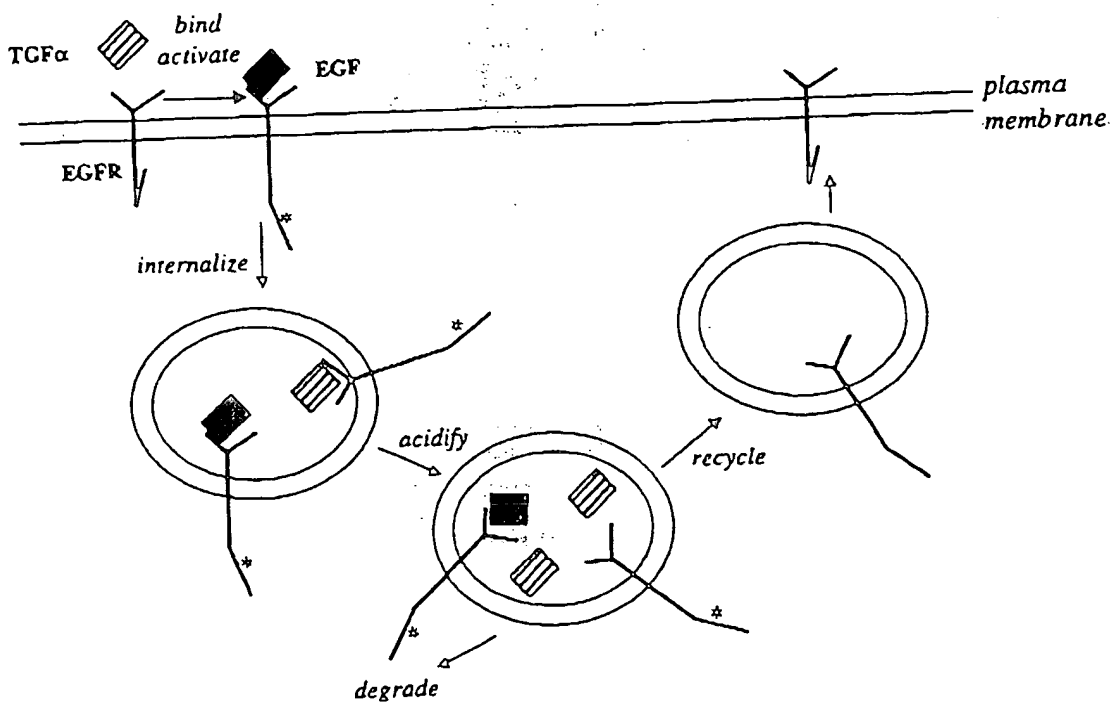

Fig. 2. Ligand-regulated trafficking and disposition of EGFR. Upon ligand binding EGFR kinase is activated and the receptor is internalized via clathrin-coated pits using a saturable, adaptin-specific mechanism. This initial action is consistent between the different ligands. In the acidified endosomal compartment, the ultimate fate of EGFR is determined by the binding properties of the ligand—EGF remains bound and directs the receptor and its bound EGF to degradation; TGFα dissociates and the receptor is recycled while the free TGFα sorts with the majority of the bulk phase to degradation. When this mechanism is saturated (at approximately 50,000 internalized EGFR), excess EGFR is recycled; in this situation EGF bound to receptor will be returned to the surface while the majority of TGFα will still be degraded with the bulk phase.

The EGFR autophosphorylation motifs are structurally similar [2] and functionally redundant, in distinction to many other RPTK. This simple architecture and flexible interchange of redundant motifs bespeaks an archetypal gene.

There are two established and one proposed EGFR variants. A splice variant proximal to the transmembrane domain generates a secreted form of EGFR which can act as a dominant-negative in experimental situations. Whether this acts in vivo as a negative titrater of signaling or as a soluble binding protein extending the life-span of EGFR ligands or acting as an extracellular sink for predeposited ligands remains to be determined. A non-ligand binding but constitutively active EGFR variant (EGFRvIII) lacking amino acids 6–273 (exons 2–7) across the first cysteine-rich domain was first reported as a tumor-specific gene rearrangement; more recent work has suggested that this rearranged gene may replicate a splice variant present during development [3]. The presence of immunologically- and biochemically-defined EGFR in the nucleoplasm has lead to suggestions of a transmembrane-negative splice variant similar to one reported for the related *k-sam* gene; however, this species has yet to be positively identified. Except for EGFRvIII in select tumor types, these EGFR variants represent minor populations observed only in limited situations.

EGFR interacts with most members of the c-erbB subfamily of RPTK. The ligand for erbB-2 is undefined while erbB-3 and erbB-4 serve as heregulin and neuregulin receptors. However, a

major function of these other receptors appears to be as downstream effectors of each other. These receptors hetero-aggregate, cross-phosphorylate, and modulate signaling from each other in specific pairings. For instance, EGFR (erbB-1) will interact with erbB-2 and erbB-3 but not erbB-4, but erbB-4 will pair with erbB-2. Of particular note is that erbB-3 lacks kinase activity, rather serving as a docking protein to recruit a broader spectrum of downstream effectors after phosphorylation by EGFR or erbB-2. Such a situation may be reiterated by the tumor-specific EGFRvIII.

3. Expression and degradation

EGFR is present on all epithelial and stromal cells as well as select glial and smooth muscle cells. It is transcribed from a TATA-less promoter as two predominant large mRNA species that differ by the extent of 3′ untranslated sequence (in humans as ~6 kb and ~9 kb species). Relatively little has been reported about transcriptional control of EGFR, though it appears to decline with cellular aging at least in dermal fibroblasts. Post-translational processing and trafficking has been extensively studied and reviewed. In polarized epithelial cells EGFR is largely restricted to the basolateral aspects, allowing for epithelial-stromal communication from fibroblast-derived TGFα and other matrix-associated EGFR ligands. This asymmetric presentation of EGFR limits autocrine signaling, as many of these epithelial organs, particularly throughout the genitourinary system, secrete copious amounts of EGF into the lumens [4].

Upon ligand binding and activation, EGFR undergo internalization via a saturable endocytic system which depends on specific adaptins and sorting nexins complexing with EGFR carboxy-terminal motifs. The fate of the receptor depends on continued occupancy and kinase activity; EGF, remaining bound in the acidic late endosomal compartment, directs EGFR to degradation whereas activation by TGFα, which displays a pH-sensitive dissociation, results in recycling. Ligand-induced internalization and degradation results in signal attenuation with net removal of either receptor (in the case of nondissociative ligands like EGF) or ligand (for dissociative ligands such as TGFα) [5] (Fig. 2). Thus, different ligands can dictate the strength and temporal lifespan of EGFR signals, thereby providing a rationale for the existence of multiple genetically-distinct ligands.

4. Biological function

EGFR is a pleiotropic signaler. The integrated biological response to EGFR activation varies from mitogenesis to apoptosis, migration to differentiation to dedifferentiation even in the same cell depending on the context, which includes cell density, type of matrix, other cytokines, and even the position within a cell colony. The molecular bases of these responses is only now being defined [6].

EGFR kinase triggers numerous downstream signaling pathways similar to other RPTK and tyrosine kinase-linked cytokine receptors. These pathways include those that involve PLCγ (phospholipase C-γ) and its downstream calcium- and PKC-mediated cascades, ras activation leading to various MAP kinases, other small GTPases such as rho and rac, multiple STAT (signal tranducer and activator of transcription) isoforms, and heterotrimeric G proteins, as well as others to a lesser extent including the phospholipid-directed enzymes PI3 kinase (phosphatidylinositol 3′-OH kinase) and PLD (phospholipase D), and the proto-oncogene cytoplasmic tyrosine kinase src. While this confusion of cascades has prevented lucid exposition of biochemical links to biological responses, a few principles are becoming clear. First, a number of signaling pathways can be shown to be required, but not sufficient for a particular response; PLCγ-mediated hydrolysis of PIP_2 (phosphatidylinositol (4,5) bisphosphate) and mobilization/activation of actin-modifying proteins is required for EGFR-mediated motility, but motility is blocked if MEK (MAP kinase kinase) signaling is abrogated [6–8]. Second, many signaling pathways contribute to multiple responses; activation of the erk MAP kinases

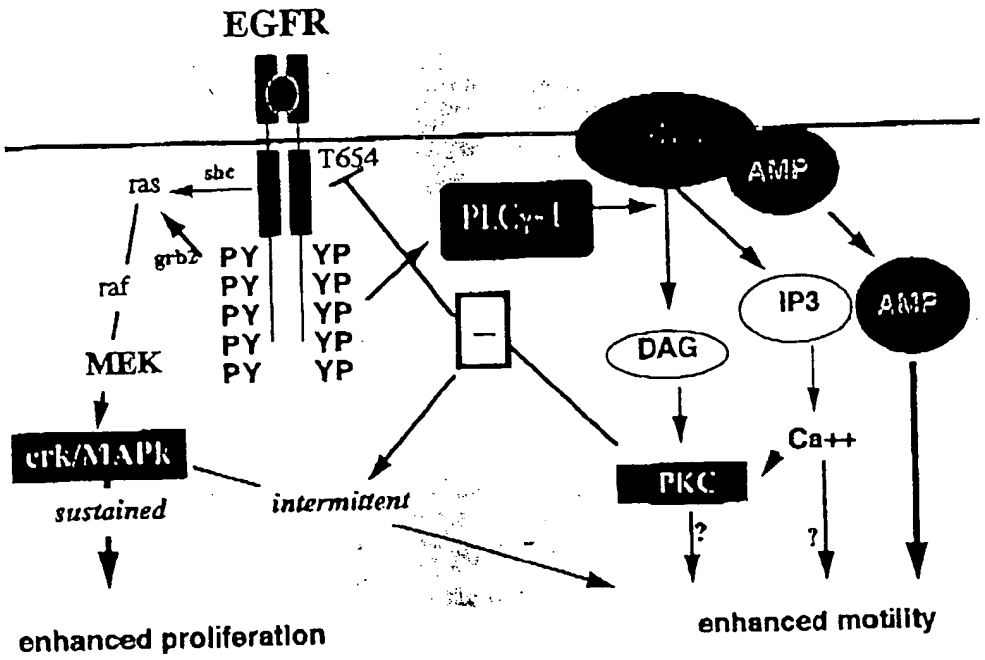

Fig. 3. Crosstalk between downstream pathways activated by EGFR. Ligand binding to EGFR initiates numerous signaling pathways. For many of these pathways, the biological outcomes have yet to be determined. Shown here are the outcomes and interplay of two robustly activated pathways, via PLCγ and ras. The PLCγ pathway not only results in positive signaling for cell motility, by mobilization of actin modifying proteins (AMP) (such as gelsolin, profilin and cofilin) and possibly via protein kinase C (PKC) and calcium-mediated events, but also feedback attenuates EGFR. PKC phosphorylation of EGFR at threonine 654 preferentially disrupts activation of the ras-MAP kinase axis. This is postulated to result in intermittent erk activity which favors motility over mitogenesis.

promotes both proliferation and migration [8]. Third, other signals, both biological and biophysical, modulate the response to EGFR activation; epithelial and stromal cells only exhibit biological responses when attached to a tensed substratum [9]. Fourth, temporal and spatial control of EGFR signaling dictates the biological outcome possibly by altering the balance between various signaling pathways; the motility-associated PLCγ pathway initiates a PKC-mediated feedback attenuation which only slightly decreases global erk activation (but likely alters localized erk populations) which shifts the response from proliferative to migratory [10] (Fig. 3).

All this begs the question of the physiological role of EGFR signaling. This has been approached by extrapolation from in vitro experiments, in vivo perturbations such as disrupting EGFR regulation by adding or blocking ligands, and, most recently, by genetic engineering. The first noted role for EGF was maturation of epithelial tissues, as evinced by precocious eye opening and tooth eruption upon injections of EGF. This developmental role has been supported by EGFR knockouts which die in the neonatal period due to severe immaturity of several epithelial organs; that the pattern and severity of the developmental retardation is dictated by the genetic background of the mice suggests that other RPTK may be recruited to subsume, at least partially, the roles of EGFR. It also was shown that postpartum milk production was enhanced by EGFR signaling, suggesting a metabolic role [11]. In adult animals, EGFR signaling has been postulated as important for organ

repair, which may be viewed as neo-organogenesis; reduction of EGF levels impairs hepatic regeneration. Throughout the genitourinary tract, high levels of lumenal EGF are proposed to stimulate repair of epithelial breaks by gaining access to basolateral EGFR.

In most of these situations the operative cellular response is assumed to be the mitogenic response to EGFR signaling. However, during development cells must proliferate, migrate and differentiate, and during repair dedifferentiation may also play a role. Parsing the individual cell responses has been confounded by the pleiotropic nature not only of EGFR signaling but also of several of the downstream effectors. Recently, we have identified PLCγ as being required for EGFR-mediated motility but not mitogenesis. Inhibiting this specific pathway in breast and prostate epithelial cells, severely retards the branching morphogenesis. This pattern is similar to mammary glands in which global EGFR signaling is abrogated by a dominant-negative EGFR [12]. This suggests that the operational response is not mitogenesis but migration. Many further investigations are needed to determine the situations in which each of the integrated cellular responses function physiologically.

5. Medical/industrial application

Control of EGFR signaling will likely provide important opportunities in three main areas—cancer treatment, organ repair, and cell production. EGFR is the receptor most often found upregulated in a wide variety of human tumors [13]. Due to its early identification as the proto-oncogene of the transforming *v-erbB* oncogene and its association with the genesis of numerous tumors, EGFR has been the target of numerous therapies, ranging from therapeutic and imaging antibodies to toxin-linked ligands to enhancement of targeting for gene therapy vectors [14]. These approaches have primarily used upregulated EGFR as a tumor-specific target based on a therapeutic index as EGFR expression is widespread; the special case of EGFRvIII deleted EGFR may, however, represent a 'true' tumor-specific antigen. In many tumors EGFR levels are not overexpressed but rather signaling is upregulated due to autocrine stimulatory loops secondary to the breakdown of cellular asymmetry and spatial segregation of EGFR and its ligands. In these cancers, the therapeutic index based on EGFR levels is not available. Rather, one needs to evaluate the biology of the tumor. Two cellular responses are considered targets: proliferation and migration. At present, EGFR-mediated but not basal migration can be approached by abrogating activation of PLCγ. We have demonstrated that in a mouse xenograft model of human prostate carcinoma, inhibition of this signaling pathway prevents tumor invasion, albeit tumor growth remains unaffected. Interestingly, this likely represent the pathological aspect of the physiological role of branching morphogenesis. EGFR-specific proliferation, which may be critical for metastatic growth in ectopic sites, currently is less accessible as most downstream targets would likely have widespread unintended toxicities to proliferative organs such as bone marrow and gastrointestinal lining. Still, EGFR-mediated downstream signals may represent a fruitful avenue for attacking tumor invasiveness as an adjunct therapy for anti-mitotic therapy or surgical/radiotherapy bulking.

EGFR modulation holds tremendous promise in promoting wound repair and limiting scarring. One can easily envision that by understanding the quantitative aspects of EGFR signaling on both proliferation and migration, new agents and materials would be developed to improve dermal wound repair, which is readily accessible to repeated and targeted 'drug' applications. During wound repair both proliferation and migration must be triggered but in the proper spatial and temporal context. Our lack of understanding of this complex orchestration likely underlies the failure of EGF and other growth factors to improve this clinical situation to date. A second use bespeaks this fine balance between augmenting and abrogating repair. Injection of high concentration of EGF results in sheep shedding their fleece and has been promoted as an alternative to shearing, though a costlier one. While current human fashion dictates against total hair loss

having a significant cosmetic market, localized depilatory action may be profitable.

One major industrial use of EGFR signaling is in the production of bio-pharmaceuticals. As genetically-engineered agents are made in mammalian cells, a limiting factor will be control of cell growth and viability. The ready availability and stability of native and recombinant EGFR ligands even in harsh conditions coupled with cross-species promiscuity make these ideal for large-scale cell cultures. Furthermore, specific alterations and modifications have been shown to alter the signaling properties to extend the bioactivity or spare cell receptors [15].

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